THE COAGULASE TEST FOR STAPHYLOCOCCI AND ITS CORRELATION WITH THE RESISTANCE OF THE ORGANISMS TO THE BACTERICIDAL ACTION OF HUMAN BLOOD[1,2]

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Advances in the study of the staphylococcus and infections caused by this micro-organism include the development of biological and serological methods for differentiating various strains of staphylococci. Numerous investigators have concluded that the coagulase reaction is one of the most practical and simple methods for differentiating pathogenic from non-pathogenic strains (1 to 7). We have arrived at similar conclusions, but it should be emphasized that the use of the terms, "pathogenic", and "non-pathogenic", express only a relative relationship. As will be pointed out, non-pathogenic strains may in rare instances, cause serious and even fatal infections.

The purpose of this report is to correlate the coagulase reaction with the resistance of staphylococci to the bactericidal action of human blood. This work is an outgrowth of a previous study in which it was shown that pathogenic strains of staphylococci were highly resistant to the antibacterial action of human bloods from healthy controls and from those recovering from severe staphylococcal infections (8). As far as we can ascertain from a review of the literature, no comprehensive reports are available concerning the relationship of coagulase production to the growth of staphylococci in human blood. In this connection, Thompson and Khorazo (9) stated that Type A strains of staphylococci grew better in human defibrinated blood than non-Type A strains.

METHODS OF STUDY

Source of strains. A total of 70 strains were studied, all isolated from human beings. Thirty-two strains were obtained from an equal number of patients having severe infections such as bacteremia, osteomyelitis, and bacterial endocarditis. Fifteen strains were sent to us by Dr. G. H. Chapman of New York City in answer to a request for human strains which he considered to be non-pathogenic. Ten strains of *S. albus* were obtained from superficial lesions and human urine. A strain of *S. albus* was cultured from the venous bloods of each of two patients with a bacterial endocarditis. Eleven strains of *S. albus* were grown from the hair and skin of normal human beings.

Cultures were grown on veal-agar slants, pH 7.8, kept in a refrigerator and transplants made every 3 weeks. Continuous transfers were made over a period of 3 months to 3 years, depending upon the time when the original culture was obtained. During the last year of study, the majority of the cultures were maintained under oil on veal-agar slants and transplants made every 6 months.

Coagulase test. The test was performed by transferring one loopful of organisms from a 24-hour culture on an agar slant to 0.5 ml. of citrated human plasma and incubating the plasma in a water bath at 37° C. The pressure of coagulum at the end of 3 or 18 hours indicated a positive test.

Bactericidal test. Eighteen-hour broth cultures were used. The broth as described by Lyons (10) consisted of veal infusion (Difco), peptone (2 per cent), glucose (0.05 per cent), and NaCl (1 per cent). Fresh, defibrinated blood from individuals free of demonstrable infections was used. Ten-fold dilutions of the broth cultures were made up to the 10^7 dilution and 0.05 ml. of each dilution was added to each of several small, pyrex glass tubes containing 0.5 ml. of defibrinated blood. The approximate number of organisms in each of the dilutions was determined by preparing pour-

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TABLE I

Correlation between coagulase and bactericidal test for staphylococci isolated from patients with severe infections

Strain number	Pigment	Coagulase test	Bactericidal test*							
			Dilution of organisms							Number of organisms per 0.05 ml. 10^7 dilution
			10^1	10^2	10^3	10^4	10^5	10^6	10^7	
1	Yellow	Positive	+	+	+	+	+	+	+	202
2	Yellow	Positive	+	+	+	+	+	+	+	331
3	Yellow	Positive	+	+	+	+	+	+	+	26
4	Orange	Positive	+	+	+	+	+	+	+	250
5	Yellow	Positive	+	+	+	+	+	+	+	56
6	Orange	Positive	+	+	+	+	+	+	+	500
7	Yellow	Positive	+	+	+	+	+	+	+	500
8	Orange	Positive	+	+	+	+	+	+	+	389
9	Orange	Positive	+	+	+	+	+	+	+	3
10	Yellow	Positive	+	+	+	+	+	+	+	160
11	Gold	Positive	+	+	+	+	+	+	+	102
12	White	Positive	+	+	+	+	+	+	+	12
13	Gold	Positive	+	+	+	+	+	+	+	215
14	Orange	Positive	+	+	+	+	+	+	+	83
15	Yellow	Positive	+	+	+	+	+	+	+	344
16	Yellow	Positive	+	+	+	+	+	+	+	190
17	White	Positive	+	+	+	+	+	+	+	185
18	White	Positive	+	+	+	+	+	+	+	64
104	Orange	Positive	+	+	+	0	0	0	0	200

* + = Growth.

plates with 0.05 ml. of the 10^6 and 10^7 dilutions. The tubes were sealed in a gas-oxygen flame and rotated for 24 hours in a box in an incubator at 37° C. At the end of this time, the tubes remained for another 24 hours in the incubator. They were then opened and the contents of each tube cultured on an agar plate to determine the presence of viable organisms.

RESULTS

All 32 strains isolated from patients having severe infections were coagulase-positive and resisted the bactericidal action of normal defibrinated blood with but one exception. Representative results are presented in Table I. It is to be noted that strains 12, 17, and 18 grew out as white colonies, and on this basis would be classified as *S. albus* cultures. In this connection, experience with strain 18 is of interest. It was isolated from the blood stream of a patient who had a fulminating and fatal infection. The presence of *S. albus* in the culture brought up the possibility of its being a contaminant. Subsequent cultures of blood yielded the same type of colonies. Repeated coagulase tests performed with different cultures of this strain gave positive results which classified the culture as belonging to the pathogenic group. After many subcultures, this strain still produces only white colonies and is resistant to the bactericidal action of whole blood. *S. albus* strains 12 and 17 were obtained from severe osteomyelitic lesions. Strain 104 was the only coagulase-positive strain that did not resist the killing effect of blood. Repeated tests with this strain, using samples of blood from several individuals, showed that a significant number of organisms were killed in every instance. However, the strain was highly pathogenic because of a lethal toxin that it produced. A laboratory worker accidentally inoculated with a broth culture of this strain expired 52 hours later. The clinical course and autopsy findings were similar to those observed by Kellaway and his associates in the Bundaberg disaster of 1928 (11).

Several veal-agar transplants were made with the 15 non-pathogenic *S. albus* cultures sent to us in 1937 by Dr. George A. Chapman. These were stated to be coagulase-negative strains. In our hands, 2 of these strains were found to be coagulase-positive, and resisted the bactericidal action of blood. The significance of these findings will be discussed shortly. The remaining cultures were all coagulase-negative, and a marked killing effect of the blood was observed for all of the strains.

Table II includes the results with a group of 12 strains of *S. albus* isolated from persons with superficial lesions and low-grade infections of the urinary tract, and from 2 patients with bacterial endocarditis. Strain 157 was coagulase-positive and no killing effect was exhibited by the whole

TABLE II

Correlation between coagulase and bactericidal tests for S. Albus *isolated from human beings*

Strain number	Coagulase test	Bactericidal test*							
		Dilution of organisms							Number of organisms per 0.05 ml. 10^7 dilution
		10^1	10^2	10^3	10^4	10^5	10^6	10^7	
150	Negative	+	0	0	0	0	0	0	53
151	Negative	+	+	0	0	0	0	0	600
152	Negative	0	0	0	0	0	0	0	15
153	Negative	+	+	0	0	0	0	0	69
154	Negative	+	0	0	0	0	0	0	131
155	Negative	+	+	+	+	0	0	0	229
156	Negative	+	+	0	0	0	0	0	656
157	Positive	+	+	+	+	+	+	+	100
158	Negative	+	+	+	0	0	0	0	23
159	Negative	+	+	0	0	0	0	0	50
160	Negative	+	+	+	0	0	0	0	251
161	Negative	+	+	0	0	0	0	0	22

* + = Growth.

TABLE III

Correlation between coagulase and bactericidal tests at intervals of three years for S. Albus *strains*

Strain number	1938									1941								
	Pigment production	Coagulase test	Bactericidal test*							Pigment production	Coagulase test	Bactericidal test*						
			Dilution of organisms						Organisms 10^7 dilution			Dilution of organisms						Organisms 10^7 dilution
			10^1	10^2	10^3	10^4	10^5	10^6	10^7			10^1	10^2	10^3	10^4	10^5	10^6	10^7	
6508	White	Negative	+	+	+	0	0	0	0	475	Yellow	Positive	+	+	+	+	+	+	+	27
6744	White	Negative	+	+	+	0	0	0	0	1021	Yellow	Positive	+	+	+	+	+	+	+	23
6707	White	Positive	+	+	+	+	+	+	+	135	Yellow	Positive	+	+	+	+	+	+	+	21
6715	White	Negative	+	+	0	0	0	0	0	116	White	Negative	+	+	+	0	0	0	0	68
6476	White	Negative	+	+	0	0	0	0	0	500	White	Negative	+	+	0	0	0	0	0	43
6756	White	Negative	+	+	+	0	0	0	0	311	White	Negative	+	+	+	+	0	0	0	31
6773	White	Negative	+	+	0	0	0	0	0	30	White	Negative	+	+	0	0	0	0	0	4
6780	White	Positive	+	+	+	+	+	+	+	135	White	Negative	+	+	0	0	0	0	0	44
155	White	Negative	+	+	+	+	0	0	0	229	Yellow	Positive	+	+	+	+	+	+	+	23
157	White	Positive	+	+	+	+	+	+	+	100	White	Positive	+	+	+	+	+	+	+	43

* + = Growth.

blood when this strain was investigated. This culture was obtained from the urine of a patient only mildly ill. In contrast, organisms from all of the 11 remaining strains were killed in large numbers. Strains 153 and 161 were isolated from the blood streams of 2 patients who had subacute bacterial endocarditis. Repeated blood cultures revealed the same species of organism to be present. Organisms from both strains grew out as white colonies; they showed a negative coagulase test; and they were killed in large numbers by the whole blood of the patients and of normal individuals. The blood streams of both individuals were sterile for long periods of time following the use of sulfonamide compounds. Cardiac failure supervened in both patients and death resulted. Post-mortem studies were carried out and clumps of cocci were present at the bases of the vegetations on the mitral valves of both cases.

Table III includes the results of the coagulase and bactericidal tests performed in 1938 and in 1941 with the same strains. During the intervening period of three years, numerous transplants had been made on veal-agar. All the strains, except 155 and 157, were procured from Dr. G. H. Chapman. The significant feature of these observations is that four of the strains (6508, 6744, 6780, and 155) showed a reversal in the results of the coagulase tests, and coincident with this, a change in their resistance to the killing power of blood. A possible explanation for these changes will be presented shortly.

DISCUSSION

While the foregoing data show a remarkably close correlation between the production of coagulase by staphylococci and the resistance of the organisms to the bactericidal action of defibrinated human blood, we do not wish to imply that coagulase, *per se,* is the factor responsible for the difference in the antibacterial action of blood against coagulase-positive and coagulase-negative strains. It is not known what effect this substance has upon the killing power of whole blood.

It was noted that some strains after many transplants exhibited a reversal of coagulase production and of their resistance to the bactericidal action of blood. It is not unlikely that the explanation for these biological differences is one of bacterial dissociation. It is well known that avirulent *S. albus* colonies may occur as variants of *S. aureus* strains (12, 13). These non-pathogenic *S. albus* variants have been shown to be coagulase-negative by Pinner and Voldrich (13). Blair also observed that a small percentage of coagulase-positive strains will lose their ability to coagulate plasma over a period of several months (14).

SUMMARY

1. The coagulase test is the simplest and most reliable method for differentiating pathogenic from non-pathogenic strains of staphylococci.

2. Coagulase-positive strains of staphylococci resist the bactericidal action of human defibrinated

blood; whereas, coagulase-negative strains are killed in large numbers, with only two exceptions in a study of 70 strains.

3. The terms "pathogenic" and "non-pathogenic", based on the results of the coagulase test, are relative since coagulase-negative strains on rare occasions may result in fatal infections. Two instances of subacute bacterial endocarditis are recorded to illustrate this.

4. Repeated subcultures of coagulase-positive strains may result in coagulase-negative strains. The reverse of this also occurs. Both phenomena are probably explained on the basis of bacterial dissociation.

BIBLIOGRAPHY

1. Fisher, A. M., The plasma-coagulating properties of staphylococci. Bull. Johns Hopkins Hosp., 1936, **59**, 393.
2. Cruickshank, R., Staphylocoagulase. J. Path. and Bact., 1937, **45**, 295.
3. Cowan, S. T., The classification of staphylococci by precipitation and biological reactions. J. Path. and Bact., 1938, **46**, 31.
4. Blair, J. E., The pathogenic staphylococci. Bact. Rev., 1939, **3**, 97.
5. Fisk, A., Technic of coagulase test for staphylococci. Brit. J. Exper. Path., 1940, **21**, 311.
6. Fairbrother, R. W., Coagulase production as a criterion for the classification of the staphylococci. J. Path. and Bact., 1940, **50**, 83.
7. Chapman, G. H., Berens, C., and Stiles, M. H., The coagulation of plasma by staphylococci. J. Bact., 1941, **41**, 431.
8. Spink, W. W., and Paine, J. R., The bactericidal power of blood from patients and normal controls for staphylococci. J. Immunol., 1940, **38**, 383.
9. Thompson, R., and Kharazo, D., Differential growth of the antigenic types of staphylococci in human blood. J. Bact., 1938, **35**, 51.
10. Lyons, C., Antibacterial immunity to staphylococcus pyogenes. Brit. J. Exper. Path., 1937, **18**, 411.
11. Kellaway, C. H., MacCallum, P., and Terbutt, A. H., Report of the Royal Commission of enquiry into the fatalities at Bundaberg, 1928.
12. Hoffstadt, R. E., and Youmans, G. P., Staphylococcus aureus: Dissociation and its relation to infection and to immunity. J. Infect. Dis., 1932, **51**, 216.
13. Pinner, M., and Voldrich, M., Derivation of staphylococcus *albus, citreus,* and *roseus* from staphylococcus aureus. J. Infect. Dis., 1932, **50**, 185.
14. Blair, J. E., The stability of biological and biochemical properties of staphylococci. J. Bact., 1938, **35**, 52.